



## Determination of the Mechanism of the Hemostatic Action of Geprocel in the Experimental Model of Thermal Damage

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**Annotation:** Evaluation of the effectiveness of hemostatic coating in necrectomy and autodermoplasty in patients with severe thermal injury, the use of Geprocel in the process of conducting research on the creation of a highly therapeutic hemostatic coating allows one-stage or delayed autodermoplasty with early necrectomy. It is convenient to change the direction of the burn disease and stop its progression, the recovery time for the integrity of the skin, the duration of inpatient treatment are reduced, which leads to a decrease in the number of infectious complications and mortality.

**Keywords:** early necrectomy, autodermoplasty, burn injury, heprocel.

**Introduction.** In the field of combustology, a number of scientific studies have been carried out in the world on the development and use of implants with a hemostatic effect, which enhance the regenerative processes of regeneration in damaged epithelial cells after autodermoplasty. For this purpose, biosynthetic and synthetic film coatings are being developed and used in clinical practice, which enhance hemostasis, reduce bleeding during necrectomy and autodermoplasty, and enhance the process of epithelization in the damaged area. Among them is a biofilm based on carboxymethyl cellulose with a high hemostatic effect, which can be used as a local preparation. In this regard, scientific research is being carried out to evaluate the effectiveness of necrectomy and autodermoplasty in patients with severe thermal injury, to create a hemostabilizing coating with high therapeutic efficacy. The creation of specially designed biodegradable hemostatic coatings during autodermoplasty for deep burns is an urgent task [1,10].

**Materials and methods.** The purpose of the experiment is to improve the results of autodermoplasty with early necrectomy using a local hemostatic agent on a model of thermal skin burn. A local hemostatic agent is a polymeric implant in the form of a powder and a film with a biosoluble hemostatic agent. It is a homogeneous film from transparent to dark in color, solubility in water at 20 ° C is 10 mg / l, soluble in most organic solutions, melting point 220 ° C. It is stable at pH 5-7, quickly decomposes in alkaline media, more resistant to acidic environments [7,9].

Model of thermal burns was performed on 18 white males weighing 180-210 g (*Rattus norvegicus f. domesticus*). In accordance with the requirements of the Declaration of Helsinki, we have created a humane approach to animals using light ether anesthesia. A technique for modeling deep thermal burns with a hot liquid with a temperature of 90-93.8 °C in rats has been developed. Under general anesthesia using halothane vapor, the rat is fixed in the abdominal cavity on the operating table under sterile conditions. The wool cover under the back was subjected to mechanical cleaning. Clean the surface 0.5 cm wider than the surface on which the skin was burned. The burn area is approximately rectangular and should cover 20% of the back. With a weight of 180-210 g, the area of the burn was 10-12 cm<sup>2</sup> or about 2.5-3x4 cm [5, 7, 8].

The study was carried out on the wound surface after necrectomy during autodermoplasty in experimental rats. Eighteen animals participated in the experiment. Experimental rats were divided into 2 groups and examined.

- In animals of the control group 1, the post-necrectomy wound and skin transplantation were performed without the hemostatic Geprocel.
- An experiment on autodermoplasty of a post-necrectomy wound and a skin graft with hemostatic Geprocel was carried out in animals of the 2nd group of the study.

Experiment 1. The animal was taken to the operating room for lunch. The area formed during the burn injury was prepared for incision using special modes of the RO-6 apparatus under inhalation anesthesia (initially under bell anesthesia, then mask anesthesia) using halothane vapors. The edges were cleaned of wool at a distance of 0.5 cm from the wound, covered with a scab. Then the hair was scraped off in the area of the right thigh, taking into account the size of the incision area. Simultaneously with capillary bleeding, necrectomy was performed to healthy tissues. In the control group (n = 9), hemostasis was performed by infusion of marl balls and coagulation in the active zones. In the main group (n = 9), hemostasis was performed by the introduction of gauze pads, coagulation in areas of active bleeding, hemostasis with the Heprocel implant. In the experimental group of animals, hemostatic powder from a cellulose product was used, which is biologically compatible with hemostasis, hygroscopic, adheres well to tissues, retains moisture, and is decomposed by hydrolysis for 3 days. It is enough to apply 10 mg of powder to the wound surface [3, 4].

During this time, the entire skin layer was sharply dissected from a pre-prepared area to remove the skin in a size corresponding to the wound formed after necrectomy. The skin was placed on a special surface and straightened with fixatives. The subcutaneous adipose tissue, skin muscles and hair follicles were then cut using magnifying optics. A prepared skin incision was made on the wound surface, where necrectomy was performed. The edges of the incision were sutured to the edges of the skin wound with separate Prolene 4/0 sutures. Due to the viscosity of the hemostatic powder, the donor graft is strengthened and does not require special fixatives.

After dermoplasty, the animals were kept in cages on a normal diet. In the first 2 days after the operation, 0.5 ml of anesthetic ipobrufen was added to the drinking water.

The process of modeling thermal burns in laboratory animals using special devices is known by various methods. However, a series of popular models related to the production of the device was not released.

We have developed a technique for modeling deep thermal burns with a hot liquid in rats. Under general anesthesia using halothane vapor, the rat is fixed in the abdominal cavity on the operating table under sterile conditions. The wool cover below the back area is subjected to mechanical cleaning. Clean the surface 0.5 cm wider than the surface on which the skin was burned. The burn area is

approximately rectangular and should cover 20% of the back. The burn area of an animal weighing 180-210 g was 10-12 cm<sup>2</sup> or approximately 2.5-3x4 cm.

The capacitive device has a hemispherical shape and has 3 holes. Hole No. 1 is designed to contact the body of the animal, the size and configuration of which is called the burn zone and the back of the animal. This hole is hermetically sealed with a thin rubber film. Hole No. 2 - 50-100 ml, depending on the size of the mo'ljallangan1 hole, intended for pouring liquid into a container with a temperature of 100 °C. Holes No. 3 up to 0.8 cm are hermetically sealed with a lead pipe, the end of which is connected to a container with a container for pouring water. The device is used as follows:

Hole #1 is placed behind the sleeping animal under anesthesia. At the agreed time, boiling water is poured into a container with a capacity of No. 2. A constant flow of water through hole No. 3 ensures that the temperature at the contact point of the animal's body is constantly maintained at the level of 90-93.8 °C. Controlling the temperature of the water at the point of contact with the skin allows you to accurately record the time of exposure, creating burn wounds similar in area and degree of tightness. A 3rd degree burn was achieved by contact count within  $9 \pm 1$  s.

**Results and its discussion.** During the experiment, we did not need to create a protective coating that was in contact with the wound surface in order for the animal burns to heal naturally. The cage in which the animals were kept was pre-treated with disinfectant solutions. In the first 3 hours after the burn, the need for pain relief in animals was not felt due to the ongoing action of halothane. On the following days, ipobrufen was added to the animals' drinking water for 3 days.

For a short time after the formation of the burn, the animals were in a state of intoxication after anesthesia. Having lost his bearings, his walking time was shortened and he could not stand well on his feet. The animals recovered within 2 hours after the operation. They ran around the corner, protecting their burns. Rapid breathing and increased heart rate are noted. The fur was not smooth. It should be noted that in the postoperative period, animals that received painkillers in combination with saline and saline to correct water and electrolyte metabolism in the abdominal cavity very slowly regain consciousness, come out of anesthesia, and have difficulty breathing. In 6 rats treated with saline (up to 10 ml) and analgesics: 3 of those treated with 50% analgin 0.3 or 0.1% - 0.1 diphenhydramine died after 3 hours, 3 animals were soaked for 2 hours due to severity their states. oxygen was needed, 2 of whom died the day after the experiment. 1 rat survived. In the next experiment, no liquid was injected into the abdominal cavity of the animals and analgesics were not injected. As an analgesic, the animals were given ipobrufen in 0.5–200 ml of water for 2 days. There were no lethal outcomes after the formation of burns in animals of this group.

Observation of the condition of the animals made it possible to establish the following (Table 1). Six hours after the formation of a third-degree burn on 20% of the body surface, the animals moved moderately and drank water. They were often in a quiet place in the corner of their cages. Contamination of the combustion surface was not allowed. The color flow of the wound in the burn area is observed, while holding it is soft and elastic. No bulge or fluid leakage was observed.

Within 3 days the animals became more active, the burn area became covered with black spots, sometimes black spots appeared in some places. The area of burns thickened, the demarcation line began to appear in the form of hyperemia. In some animals, contamination manifested itself in the form of moistening of the wound surface and thickening of the skin over the burn zone. Animals became more mobile and began to consume water and feed. Weight loss up to 20% depending on the initial condition.

After 7 days, the animals became more mobile, began to consume water and feed well. The burn site is covered with a dark-colored scab, hard, inelastic. The area of the burn is significantly reduced to 80% of the original appearance. In almost all animals, scab seeding was observed in the form of a mucous

membrane, slightly separated from the granulation focus, covered with an opaque appearance. No eschar separation was observed during active bleeding from the wound. Weight loss up to 15% depending on the initial condition.

**Table 1. Dynamics of body weight in rats of the main and control groups at different times of the experiment (d)  $M \pm m$**

№	Check time	Dynamics of animal weight ( g).		P
		base	the control	
1.	until it burns	198.44 $\pm$ 1.72 (n = 35)	196.42 $\pm$ 1.86 (n = 18)	> 0.05
2.	1st day	195.29 $\pm$ 1.63 (p = 35)	193.15 $\pm$ 1.79 (p = 27)	> 0.05
3.	3rd day	191.20 $\pm$ 1.60 (p = 35)	189.42 $\pm$ 1.79 (p = 27)	> 0.5
4.	5th day	189.68 $\pm$ 1.48 (p = 35)	187.19 $\pm$ 1.70 (p = 27)	> 0.05
5.	7th day	189.11 $\pm$ 1.49 (p = 35)	186.53 $\pm$ 1.69 (p = 27)	> 0.05
6.	12th day	190.02 $\pm$ 1.57 (p = 35)	187.03 $\pm$ 1.75 (p = 27)	> 0.05
7.	20th day	195.11 $\pm$ 1.80 (p = 10)	179.66 $\pm$ 2.45 (p = 10)	<0.005
8.	30th day	200.50 $\pm$ 1.32 (p = 5) *	182.50 $\pm$ 2.22 (p = 5) *	<0.005

\* - Significantly relative to start time

Within 14 days after incineration, the animals are more active, drinking water more actively. It is noted that 5-10% of body weight gain occurs on the 3rd day after the burn. The resulting burn area was covered with hard scabs with signs of contamination. The area is reduced by up to 30%. In most animals, the skin of the wound is removed without bleeding, under which actively healing tissue with a small detachment, covered with a film, is found.

On the 21st day after burning, the animals are more mobile. There was no weight gain compared to day 14. The burn area decreased to 40% and began to have an elongated stellate appearance. In most animals, the place of the skin that has moved away from the wound is new, more closely adjacent to the wound, with difficult-to-remove, pinpoint symptoms of hemorrhage.

On the 30th day after burning, the animals are active. The tendency to shrink on the surface of the burn continues to shift - it began to take on an elongated shape. Where the bark has moved and formed, there is a scar. The weight of the animals has stabilized. By day 40, all animals are healthy. In most animals, a scar formed in the form of an irregular bulge, accounting for 20% of the initial burn injuries. In 30% of rats, the wound skin in some areas of the wound was preserved, hard, well adhering to the wound. The weight of the animals returned to their original position. On the 60th day of the experiment, the wound in all animals completely healed with the formation of a long white scar, no hair loss was observed.

In laboratory rats, in almost all cases, natural processes of healing of deep skin burns are observed when microbial flora is added. According to histological studies, as well as after the formation of experimental burns on the skin, observation of the animals showed the onset of infection from the 3rd day after the injury. On the 3rd day, signs of wound contamination were noted in 50% of experimental animals. Limited burns (up to 20% of the body surface area) and clinical appearance differ little from the normal state of the animals. The animals remained active, moved freely around the cages, ate and drank. At the time of examination, the wound was covered with scars, hard; in most animals, separation from the wound was not observed. In 10% of cases, the surface of the eschar was rough and colored, with little separation from the wound. Symptoms of histological neutrophilic leukocyte infiltration. On the 3rd day, an important sign of a burn injury is the appearance of a demarcation line, which appears as a light red border around the perimeter of the burn. At a much earlier stage, such a border between the burn area and healthy tissue was not macroscopically formed.



On the 4th day or more after the onset of a deep skin burn, almost all experimental animals showed signs of infection of the wound under the scab, and necrectomy against the background of infection did not allow dermoplasty due to the risk of transplantation. In experimental animals, the process of contraction of the existing skin defect under the scab continued. The gradual decrease in the size of the lesion led to a gradual narrowing of the area as the scab moved from the peripheral zones. Depending on the size of the damaged area and the degree of contamination of the wound, the healing process lasted up to 60 days. Upon completion of the inflammatory and reparative processes, the final stage ended with the formation of an elastic, almost imperceptible elongated scar, more often in the form of a broken line or zigzag. Scars in our study were not observed with hypertrophic or keloid transformation characteristic of this type of laboratory animals.

Thus, to accelerate the healing of burn injuries in laboratory rats, it is optimal to perform autodermoplasty with nervectomy on the 3rd day after the burn, when wound contamination is minimal. Since the demarcation process was not clearly developed, it was difficult to define the boundary of the skin incision. The depth of the tissue incision was determined by the degree of tissue bleeding and passed into the muscle. The dermoplasty procedure performed later was accompanied by contamination and migration of the skin graft.

In our experimental studies, the day of necrectomy - day 3 - was confirmed by the following factors: deep skin burns - up to 20% of the body surface area, no need for parenteral fluids, since the animals lost their natural fluid intake after anesthesia. filled with drinking water. In our experiments, in 20% of cases of burns of the posterior surface, no lethal outcomes were observed. The presence of animals in natural conditions and the absence of dressings after the formation of a burn caused almost 100% seeding of the wound on the 4th day, which made it impossible to perform primary plasty after necrectomy.

On the postoperative day, the animals were moderately active and tried to avoid the area of surgical intervention. We added ipobrufen to the water for 2 days to relieve postoperative pain. Protective links were not set.

9th day after autodermoplasty. On the right contour of the wound, a living area of the transplanted skin is visible. The left quadrant is still covered with eschar, which contains a partially necrotic part of the graft. The 2 animals with partial skin necrosis also had dense scabs covering approximately 50% of the graft surface. No other signs of contamination were found. Active without restrictions in the affected area of animals. 2 Complete necrosis and contamination occurred in the animal skin graft. The wound was completely covered with uneven rough scabs.

The edges of the wound are infiltrated, redness is noted. There are no injuries or breakups. Animals are very active.

In 2 out of 9 animals of the control group (30%), a complete complete restoration of the defect was observed with a complete reduction of the skin autograft due to a partial reduction (up to 30%) of the defect area. Skin sutures were removed on the 8th day. Hair growth was observed at the site of the autograft . Within 7 days after the end of the donor area, a thin elastic scar remained without signs of hypertrophy and inflammation.

In 7 out of 9 animals in the group after necrectomy and autodermoplasty, the defect completely healed on the 21st day after dermoplasty, the graft was completely completed on the 7th day after the operation, partial necrosis was observed on the 14th day. No deaths were reported.

In the main group of animals, the operation method was also performed on the 3rd day after the formation of deep skin necrosis in the back and groin area using a new apparatus and boiling water. The difference between the surgical intervention in rats of this group was the use of the hemostatic

drug Geprocel to stop bleeding after the incision of necrotic skin and subcutaneous structures up to the fascia and muscles. After applying a hemostatic film up to 50 µm thick from a cellulose product, the bleeding completely stopped and became shiny due to the adhesion of the film to the wound surface. Skin removal and treatment were the same as in the control group. It was noted that when closing the wound defect, the adhesion of the skin flap to the underlying wound was significantly better than in the control group. In the postoperative period, additional ligating and restricting devices were not applied. The donor area, similar to the animals in the control area of the skin, was sutured.

3rd day after surgery a. In the main series of operated animals, a positive trend in the survival rate of skin flaps was noted. 5 rats in good condition, active. In constant motion in a cage. The fur is soft and shiny. Skin flap of normal color, soft elastic. The condition of the seams on the skin is good. Along the suture line there are small areas covered with a scab in the form of a strip up to 1 cm wide. There is no detachment from injury. The donor site wound is also clean, the sutures are in good condition, there are no signs of contamination. Against the background of a good general condition in 1 rat of the same series, scabs appeared on the surface of the skin graft along the suture line, occupying less than 50% of the transplanted skin with a width of 2 to 4 mm. The area of the skin graft, not covered with scabs, of normal color, soft, elastic. There are no signs of contamination. All rats in the control group showed no signs of contamination of the donor skin.

**Conclusion.** The recently developed technology of autodermoplasty using the hemostatic drug Geprocel has significantly improved the results of surgical treatment of deep burns. Local application of the hemostatic drug Geprocel on the wound during autodermoplasty after necrectomy provides rapid engraftment, rapid and complete (100%) healing of the skin lesion. The use of Geprocel in autodermoplasty after primary tangential necrectomy provides complete hemostasis and leads to good revascularization with free skin lesions. Early activation of animals as a result of early necrectomy and the use of the drug Geprocel and an increase in their weight is manifested in the form of a faster relief of clinical signs of burn intoxication.

The use of Geprocel allows one-stage or delayed autodermoplasty with early necrectomy, contributes to the relief of a burn and stops its progression, restores the integrity of the skin lining, reduces the duration of inpatient treatment, reduces the number of infectious complications and mortality.

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